Investigation of infection

Dr. Nashwan Mansoor

Investigation of infection

❖ **The aims of investigating a patient with suspected infection are :-**

- **Confirm the presence of infection.**
- **Identify the specific pathogen(s).**
- **Identify its susceptibility to specific antimicrobial agents in order to optimize therapy.**

❖ **The presence of infection may be suggested by ;**

- **Identifying proteins that are produced in response to pathogens as part of the innate immune.**
- **Acute phase responses.**

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❖ **Pathogens may be detected :-**

- **Directly (e.g. by culturing a normally sterile body site).**

- **Indirect detection by identifying the host response to the organism.**

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❖ **Tests used to diagnose infection:-**

➢ **Non-specific markers of inflammation/infection :- e.g.**

✓ **WCC.**

✓ **CRP.**

✓ **Procalcitonin.**

✓ **Serum lactate.**

✓ **Cell counts in urine or(CSF).**

✓ **CSF protein and glucose.**

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❖ **Tests used to diagnose infection:-**

➢ **Direct detection of organisms or organism components :-**

✓ **Microscopy.**

✓ **Detection of organism components (e.g. antigen, toxin).**

✓ **Nucleic acid amplification (e.g. polymerase chain reaction).**

➢ **Culture of organisms :- ± Antimicrobial susceptibility testing.**

➢ **Tests of the host's specific immune response :-**

✓ **Antibody detection.**

✓ **Interferon-gamma release assays (IGRA).**

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❖ **How to provide samples for microbiological samplings :-**

➢ **Communicate with the laboratory**

✓ **Discuss with laboratory staff before collection.**

✓ **Communication is key to optimizing microbiological diagnosis.**

✓ **Discuss with laboratory staff before hand than to risk diagnostic delay by inappropriate sampling or sample handling.**

➢ **Take samples based on a clinical diagnosis**

✓ **Sampling in the absence of clinical evidence of infection is rarely appropriate.**

➢ **Use the correct container**

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❖ How to provide samples for microbiological samplings :-

➢ Use the correct container

✓ **Certain tests require proprietary sample collection equipment.**

➢ Follow sample collection procedures

✓ **Failure to follow sample collection instructions can result in**

- **False-positive (e.g. contamination of blood culture samples).**
- **False-negative (e.g. collection of insufficient blood for culture) results.**

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❖ **How to provide samples for microbiological samplings :-**

➢ **Label sample and request form correctly according to local policies,**

➢ **Use appropriate packaging**

✓ **Close sample containers tightly and package securely (usually in sealed plastic bags).**

✓ **Attach request forms to samples but not in the same compartment.**

➢ **Manage storage and transport**

✓ **Transport samples to the microbiology laboratory quickly.**

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❖ Direct detection of pathogens

- ➢ **Provide rapid results and enable detection of organisms that cannot be grown easily on artificial culture media, such as Chlamydia spp.**

- ➢ **Provide information on antimicrobial sensitivity, e.g. Mycobacterium Tuberculosis.**

❖ Detection of whole organisms

- ➢ **Whole organisms are detected by examination of biological fluids or tissue using a microscope.**

- ❑ **Bright field microscopy.**
- ❑ **Dark field microscopy.**
- ❑ **Electron microscopy.**
- ❑ **Flow cytometry.**

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❖ Detection of components of organisms

➢ **Components of microorganisms detected for diagnostic purposes include;-**

- ✓ **Nucleic acids.**
- ✓ **Cell wall molecules.**
- ✓ **Toxins.**
- ✓ **Other antigens.**

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❖ **Detection of components of organisms**

✓ **Nucleic acid amplification tests**

- **Specific sequences of microbial DNA and RNA are identified using a nucleic acid primer.**
- **The most commonly used amplification method is the polymerase chain reaction (PCR).**
- **Reverse transcription (RT) PCR is used to detect RNA from RNA viruses (e.g. hepatitis C virus and HIV-1).**
- **In multiplex PCR, multiple primer pairs are used to enable detection of several different organisms at once.**
- **NAATs are the most sensitive direct detection methods and are also relatively rapid.**

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❖ **Detection of components of organisms**

✓ **Nucleic acid amplification tests**

- **Used widely in virology.**
- **In bacteriology, PCR is used to examine CSF, blood, tissue and genital samples, and multiplex PCR is being developed for use in faeces.**
- **Helpful for microorganisms that cannot be readily cultured.**
- **Increasingly used in mycology and parasitology.**

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- ❖ **Detection of components of organisms**
  - ✓ **Nucleic acid amplification tests**

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❖ Culture :-

- Microorganisms may be detected and further characterized by culture from clinical samples.
- In vitro culture of bacteria and fungi is used to;
  - ✓ Confirm the presence of pathogens.
  - ✓ Allow identification.
  - ✓ Test antimicrobial susceptibility.
  - ✓ Subtype the organism for epidemiological purposes.
- limitations: results are not immediate, even for organisms that are easy to grow, and negative cultures rarely exclude infection.

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❖ Culture :-

- Organisms such as Mycobacterium tuberculosis are slow-growing, typically taking at least 2 weeks, Even in rapid-culture systems.
- Mycobacterium leprae and Tropheryma whipplei, cannot be cultivated on artificial media.
- Others (e.g. Chlamydia spp. and viruses) grow only in culture systems, which are slow and labor-intensive.
- The terms ‘bacteremia’ and ‘fungaemia’ describe the presence of bacteria and fungi in the blood.
- Blood-stream infection’ is the association of bacteremia/fungaemia with clinical evidence of infection.

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- **Culture :-**

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❖ Indirect detection of pathogens :-

- **Used to detect the host's immune (antibody) response to a specific microorganism.**
- **Can enable the diagnosis of infection with organisms that are difficult to detect by other methods or are no longer present in the host.**
- **The term 'serology' describes tests carried out on serum and includes both antigen (direct) and antibody (indirect) detection.**
- **Organism-specific antibody detection is applied mainly to blood.**
- **Results are typically expressed as titers.**

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❖ Indirect detection of pathogens :-

- **‘Seroconversion’ is defined as either a change from negative to positive detection or a fourfold rise in titer between acute and convalescent serum samples.**

- **An acute sample is usually taken during the first week of disease and the convalescent sample 2–4 weeks later.**

- **Earlier diagnosis can be achieved by detection of immunoglobulin M (IgM) antibodies, which are produced early in infection.**

- **A limitation of these tests is that antibody production requires a fully functional host immune system, so there may be false-negative results in immunocompromised patients.**

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❖ Indirect detection of pathogens :-

❑ Enzyme-linked immunosorbent assay

- The principles of the (ELISA, EIA) are in assays rely on linking an antibody with an enzyme that generates a color change on exposure to a chromogenic substrate.
- Configurations allow detection of antigens or specific subclasses of immunoglobulin (e.g. IgG, IgM, IgA).
- ELISA may also be adapted to detect PCR products.

❑ Immunoblot (Western blot)

- Microbial proteins are separated and transferred (blotted) on to a nitrocellulose membrane, which is incubated with patient serum.
- Binding of specific antibody is detected with an enzyme–anti-immunoglobulin.
- A highly specific test, which may be used to confirm the results of less specific tests.

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❖ Indirect detection of pathogens :-

❑ Immunofluorescence assays

- ➢ Indirect immunofluorescence assays (IFAs) detect antibodies.
- ➢ Any virus-specific antibody present in the serum binds to antigen.

- ➢ Fluorescence is visualized using a microscope.
- ➢ This method can also detect organisms in clinical samples, using a specific antibody.

❑ Complement fixation test

- ➢ Any specific antibody in the serum will complex with the antigen.

- ➢ The degree of erythrocyte lysis reflects the remaining complement and is inversely proportional to the quantity of the specific antigen–antibody complex present.

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❖ Indirect detection of pathogens :-

❑ Agglutination tests

- When antigens are present on the surface of particles and cross-linked with antibodies, visible clumping (or 'agglutination') occurs.
- The test lacks sensitivity and specificity but is still used to diagnose rickettsial infection in resource-limited settings.
- The Widal test reaction uses a suspension of Salmonella typhi and S. Para typhi 'A' and 'B', treated to retain only 'O' and 'H' antigens.
- Antigens are kept to detect corresponding antibodies in serum from a patient suspected of having typhoid fever.
- The test is not specific but is still used in some parts of the world.

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❖ Indirect detection of pathogens :-

❑ Antibody-independent specific immunological tests

- **The interferon-gamma release assay (IGRA) is being used increasingly to diagnose (LTBI).**
- **IGRA cannot distinguish between latent and active tuberculosis infection.**
- **Use only in countries where the background incidence of tuberculosis is low.**

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❖ Antimicrobial susceptibility testing :-

- If growth of microorganisms in culture is inhibited by the addition of an antimicrobial agent, the organism is considered to be susceptible to that antimicrobial.
- Bacteriostatic agents cause reversible inhibition of growth.
- Bactericidal agents cause cell death.
- Fungistatic/fungicidal are equivalent for antifungal agents.
- Virustatic/virucidal for antiviral agents.

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❖ Antimicrobial susceptibility testing :-

- **The lowest concentration of antimicrobial agent at which growth is inhibited is the minimum inhibitory concentration (MIC)**

- **The lowest concentration that causes cell death is the minimum bactericidal concentration (MBC).**

- **If the MIC is less than or equal to a predetermined breakpoint threshold, the organism is considered susceptible.**

- **If the MIC is greater than the breakpoint, it is resistant.**

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❖ Antimicrobial susceptibility testing :-

➢ **The relationship between in vitro antimicrobial susceptibility and clinical response is depends on;**

- ✓ **Immune status.**
- ✓ **Pharmacokinetic variability.**
- ✓ **Comorbidities that may influence pharmacokinetics or pharmacodynamics.**
- ✓ **Antibiotic dosing.**
- ✓ **MIC/MBC.**

GOOD LUCK